CANTALANIN-A. A NEW SAPONIN FROM THE LEAVES OF *AGAVE CANTALA*

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ABSTRACT.—The leaves of *Agave cantala Roxb.* yielded a new steroidal saponin named cantalanin-A, which is a glycoside of hecogenin with two glucose molecules. The structure of cantalanin-A has been established as O-α-D-glucopyranosyl-(1→6)-O-α-D-glucopyranosyl-(1→3)-hecogenin.

The leaves of *Agave* species (Agavaceae) yield a valuable fiber, which is mostly used for making ropes, cordage and twine. It has also been used for the manufacture of coarse fabrics which are commonly used for binders, fishing nets, hamlocks and doormats.

A review of the literature showed that leaves of different *Agave* species contain a number of steroidal saponins and sapogenins, while *Agave cantala* has been reported to contain hecogenin (1, 2). As no study seems to have been made of the saponins of *A. cantala*, this work was undertaken. The present paper describes the elucidation of the structure of saponin extracted from the leaves of this plant.

Leaves of *A. cantala*, when extracted with ethanol and treated by the method of Varshney (3), gave a cream colored product, which on tlc examination showed one major spot and some impurities. The major compound was purified by column chromatography to yield a light cream powder, mp 210–213°, $[\alpha]_D$ +75.21° (c, 1% MeOH), which was named cantalanin-A.

Cantalanin-A on acid hydrolysis yielded hecogenin (mp 263–264°). The hydrolysate obtained was neutralized and deionized. Paper chromatography, glc, and hplc showed it contained glucose as the only sugar. From the glc data, the molar ratio of aglycone to glucose was found to be (1:2).

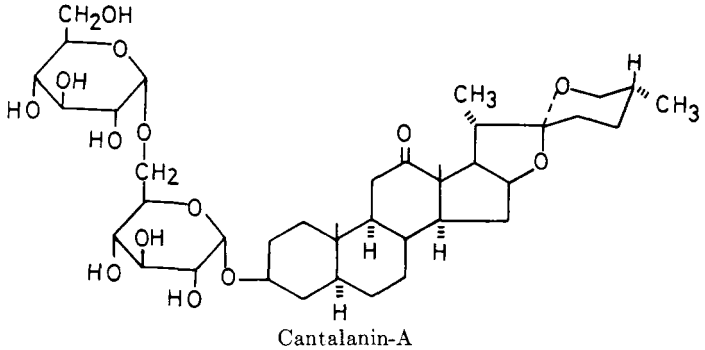
In order to determine the sequence and mode of linkage, cantalanin-A was completely methylated (4), and the permethylated saponin was subjected to methanolysis (5). The resulting methylglycosides of the methylated sugars were identified by glc as:

(I) methyl 2,3,4,6-tetra-*O*-methyl-α, β-D-glucoside and

(II) methly 2,3,4-tri-*O*-methyl-α, β-D-glucoside

Cantalanin-A, on treatment with β-glucosidase, did not liberate any sugar, indicating that none of the glucose residues has a β-linkage.

Calculation of the molecular rotation of the saponin, based on Klyne's (6)



Cantalanin-A

rule, also supports the α-linkages of the sugars. The observed $(M)_D$ value of +567.1 is in reasonable agreement with the calculated $(M)_D$ value of +573.9.

The ir spectrum of cantalanin-A shows absorption bands at 860, 900, 918 and 980 cm^{-1} indicating that the ring F is closed and no sugar attachment is possible at C–26. Thus the disaccharide moiety is attached to the C–3 OH group of hecogenin, as this is the only possible position of attachment. On the basis of the above results, the structure of cantalanin-A is *O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl (1→3)-hecogenin.

EXPERIMENTAL[1]

ISOLATION AND PURIFICATION OF SAPONIN.—Dried and powdered leaves of *Agave cantala* Roxb. collected from plants growing in Srinagar-Garhwal, were defatted with light petroleum ether (40–60°) and extracted with 95% ethanol. The ethanol extract was concentrated under reduced pressure and was successively extracted with petroleum ether, ether, chloroform, and acetone. The residue was dissolved in a minimum quantity of methanol and then added to several large volumes of ether-acetone (1:5). The precipitated material was separated each time. The precipitate gave all the tests indicative for steroidal saponin. The material was purified by passage through a column of silica gel with chloroform-methanol-water (65:30:10) as eluant.

Cantalanin-A (100 mg) was hydrolyzed with 2N aqueous sulfuric acid (100 ml) by heating first on a water bath for 30 min. and then by refluxing for three hours.

ISOLATION OF SUGARS.—The hydrolyzate obtained by acid hydrolysis of the saponin was neutralized with freshly precipitated barium carbonate and then deionized by passage through columns of Amberlite IR 120(H^+) and IRA 400 ion-exchange resins. The sugar solution was evaporated to dryness under reduced pressure to give a brown syrup. To the dried sugar (10 mg), pyridine (1 ml), hexamethyldisilazane (0.5 ml) and trimethylchlorosilane (0.5 ml) were added. The reaction mixture was shaken vigorously for 15 minutes to give the silyl derivative of the sugars. One μl of this solution was analyzed by glc.

METHYLATION OF SAPONIN.—Sodium hydride (50%) dispersed in oil (250 mg) was suspended in DMSO (10 ml) and kept in an oil bath at 80° for 1 hr. A solution of saponin (75 mg) in DMSO (5 ml) was then gradually added and the mixture was kept for one hr with constant stirring. It was cooled in ice water, and methyl iodide (3 ml) was added dropwise. The mixture was stirred for 6 hr and the methylated product was isolated in the usual manner (4). The latter showed the absence of hydroxyl absorption in the ir spectrum indicating complete methylation.

METHANOLYSIS OF METHYLATED SAPONIN.—Methylated cantalanin-A (60 mg) was treated with 3% methanolic hydrogen chloride (10 ml) in a sealed tube at 70° for 5 hr. The sealed tube was cut open, and the contents were neutralized with silver carbonate. The mixture was filtered and the methanol evaporated under reduced pressure.

ENZYMATIC HYDROLYSIS.—Cantalanin-A (10 mg) was dissolved in sodium acetate buffer solution (10 ml, pH 5) and β-glucosidase (3 mg) was added to it. The mixture was then sealed in a glass tube and kept at 37°. The tube was cut open after 50 hr. The solution was deionized and concentrated to a syrup. No sugars were detected when the latter was chromatographed.

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[1]Specific rotations are equalibrium values. All evaporations were carried out under reduced pressure at 40–50°. Whatman filter paper No. 1 was used for paper chromatography, and the solvent systems were either *n*-butanol-pyridine-water (6:4:3) or ethylacetate-pyridine-water (22:10:1). The locating reagent used was either *p*-anisidine hydrochloride or alkaline silver nitrate reagent. The tlc study of the saponin was carried out on silica gel layers using *n*-butanol-acetic acid-water (4:1:5) or chloroform-methanol-water (65:30:10). The spots were revealed by spraying with either 5% sulfuric acid or cinnamaldehyde reagent. Glc was carried out on a Perkin-Elmer model 3920 B using a column packed with 8% OS–138 on chromosorb-W (NAW), nitrogen as the carrier gas and with FID. Hplc was carried out on Waters Associates Liquid Chromatograph model 440 on a μ Bondapak/carbohydrate column with acetonitrile-water (80:20) as the solvent system.